PHOTOREACTIVATION IN COLPIDIUM COLPODA[1]

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Photoreactivation or reversal of the injurious effects of ultraviolet radiations (UV) by subsequent illumination with visible light has been observed in a variety of forms (for references, see Kelner, 1951 and Wells and Giese, 1950) but only one species of protozoan has been tested, namely *Paramecium aurelia* (Kimball and Gaither, 1951). Although considerable information has accumulated on photoreactivation and wave-length dependence of photoreactivating light, temperature dependence, photoreactivation time span etc. have been studied, the mechanism by which photoreversal occurs is still obscure. Additional data on forms other than those already studied may therefore be useful for this purpose. The present paper presents information of this kind on *Colpidium colpoda*, a ciliate protozoan. This form was chosen because it has a relatively high and regular division rate and is sufficiently large to be handled with ease and to be seen and counted with low powers of the dissecting microscope even with red light. Cultures were grown at 26° C. in 0.05% lettuce extract seeded the day before with *Pseudomonas ovalis*, in small tubes made from 0.4 mm. soft glass tubing, as described in detail elsewhere (Giese, 1945b).

Methods

The UV radiation from a quartz mercury arc run at atmospheric pressure and at 200 volts, 2.2 amperes, was passed through a natural quartz monochromator to resolve the light. The desired wave-length was focused manually on a slit between two razor blades. The light passing through the slit was reflected onto a horizontal quartz cell by a right angle quartz prism. Visible light of various wave-lengths was obtained by passing through a monochromator radiations from a GE medium pressure mercury arc. In each case the intensity of the light was measured by a thermopile calibrated against standard lamps (U. S. Bureau of Standards).

After irradiation with UV the colpidia were always handled in darkness or in dim red light from darkroom safety lights tested spectroscopically to make sure that only red light was transmitted, since some defective red bulbs transmit parts of the entire spectrum. Observations and counts were made under low power (× 6.6) with a B. and L. dissecting microscope. The number of individuals in a tube was counted three times daily and averaged for a given series. From these data the division rate was determined as described in previous reports (Giese, 1939). The light used for illuminating the colpidia while counting them was enclosed in a black cover and the beam was passed through a water cell and a Corning filter #2412 which transmits mainly wave-lengths 7400–6150 Å (cut off at λ5950 Å).

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Experimental

The first experiments were designed to determine whether photoreactivation occurred. The colpidia were irradiated with a series of dosages of UV at λ2654 Å and then treated with blue light of λ4350 Å. It was apparent in the first experiments that photoreactivation of considerable degree had occurred. The nature of the experiments and the analysis of the data are illustrated in Figure 1. The dosages of λ2654 Å tried were 750, 1000, 1500 and 3000 ergs/mm². After the colpidia had been treated with a dosage of 3000 ergs/mm². at λ2654 Å, about 70% died and the remainder were greatly retarded in division. Yet in a sample of the same batch treated with a dosage of 113,000 ergs/mm². of blue light after the UV, all individuals survived and divided, the degree of retardation corresponding approximately to the amount found after somewhat less than 1000 ergs/mm². of the UV alone. Colpidia treated with UV plus visible in all cases act as though they had been given much

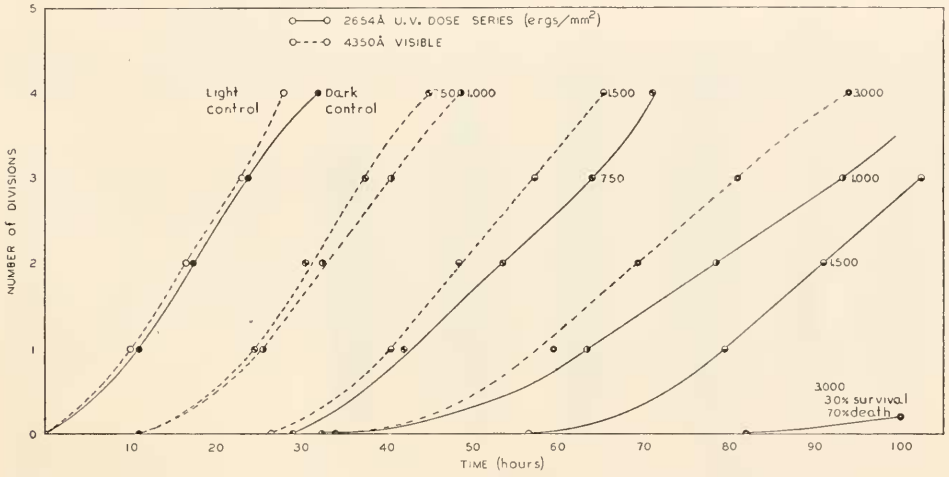

Figure 1. Effect upon division delay in Colpidium of various dosages of λ2654 Å with and without subsequent exposure to 56,900 ergs/mm². of blue light (4350 Å).

lower dosages of UV alone (see Kelner, 1949, dose reduction principle). Colpidia were exposed to a dosage of 1000 ergs/mm². of UV in all subsequent experiments because it measurably retards division, yet recovery of normal division rate occurs within a reasonable time (several days). When larger dosages are used the experiments last too long a time; it is then possible that various factors other than irradiation become limiting. Thus the medium containing bacteria deteriorates as a growth medium for Colpidium in 6–8 days. While transfer from old medium to new can be performed it involves undesirable handling of the animals.

The second series of experiments concerned itself with the amount of blue light necessary for maximal photoreversal of retarded division of Colpidium after UV treatment. In Figure 2 are given data for a series of experiments in which colpidia were irradiated with a dosage of 1000 ergs/mm². at λ2654 Å and were then illuminated with blue light (λ4350 Å) of approximately the same intensity but of dif-

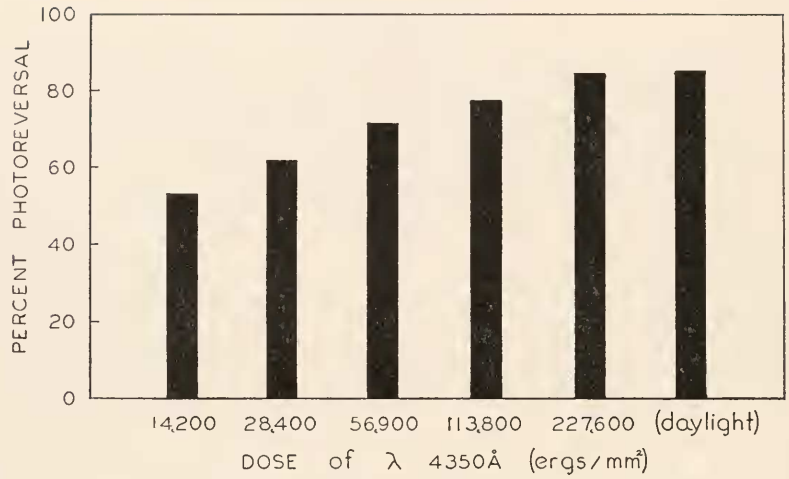

FIGURE 2. Effect upon division delay in Colpidium of various dosages of blue light (4350 Å) after a dosage of 1000 ergs/mm². of λ2654 Å. In one case the effect of daylight is compared to the effect of blue light.

ferent dosages. A progressively increasing degree of photoreversal occurs with increasing doses of blue light. However, even a 16-fold increase from the smallest to the largest tested increased the average photoreactivation only from 56% to 84%. Similar results on bacteriophage have been reported by Dulbecco (1950). The possibility that the mixture of visible wave-lengths as obtained in daylight might be more effective than blue light was tested in an experiment in which the UV-treated colpidia were exposed to indirect daylight for four hours on a bright day.

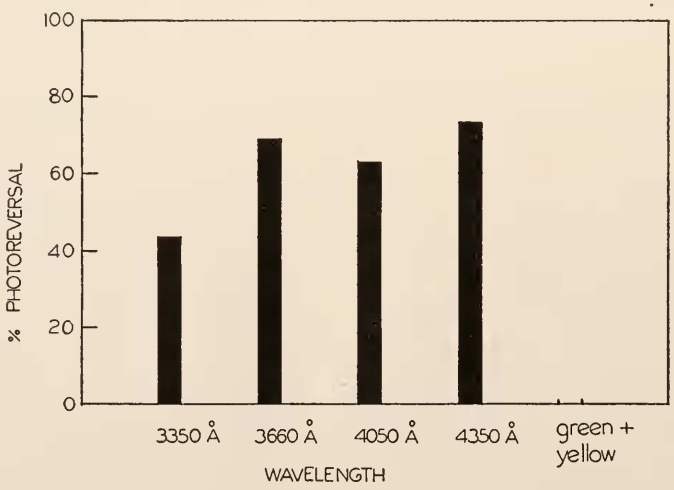

FIGURE 3. Effect upon division delay in Colpidium of a dosage of 56,900 ergs/mm². of various wave-lengths of long ultraviolet and visible light after a dosage of 1000 ergs/mm². of λ2654 Å.

The last bar in Figure 2 shows that reactivation after this large dosage of daylight is no greater than after 227, 600 ergs/mm². of blue light. While daylight might serve for photoreactivation studies, it is variable qualitatively and quantitatively; therefore a measured dosage of blue seemed preferable. While maximal photoreactivation with blue light might be desirable in some experiments, the time involved in giving a dosage of 227,600 ergs/mm². of blue light was too long to be practicable for most experiments; therefore a dosage of 56,900 ergs/mm². was settled upon as giving an adequate and satisfactory photoreversal, only 12% short of the maximal achievable.

To determine whether light other than blue might be more efficient for photoreversal in colpidia, the effect on photoreversal of several other wave-lengths (long ultraviolet: λ3350, 3660; violet, 4050; and yellow-green) available in the spectrum

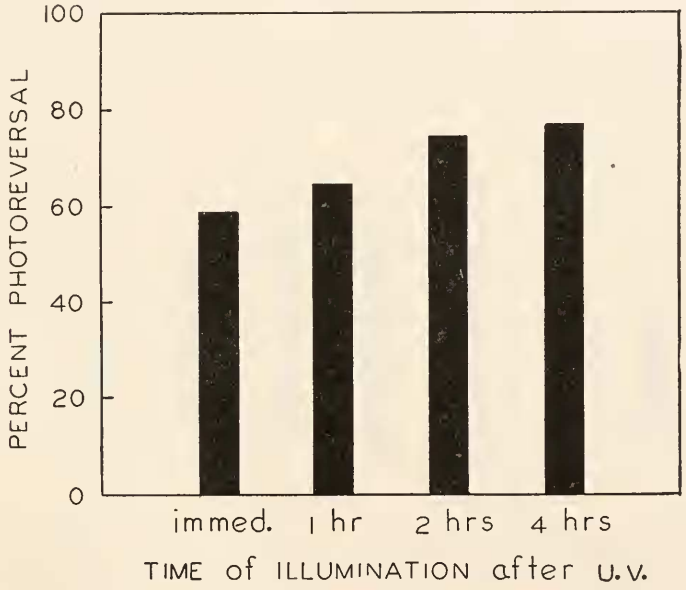

FIGURE 4. Effect of time lapse upon the degree of photoreactivation of division of Colpidium. The colpidia were given a dosage of 1000 ergs/mm². of λ2654 Å followed by a dose of 56,900 ergs/mm². of blue light (4350 Å) at the time specified.

of the mercury arc was compared with blue. The data are summarized in Figure 3. In all cases colpidia were first exposed to 1000 ergs/mm². of UV of λ2654 Å usually followed by 56,900 ergs/mm². of the photoreactivating light. It will be observed that the long UV (3350 and 3660 Å), the violet (4050 Å), and the blue (4350 Å) are quite effective for photoreversal. On the other hand yellow-green has no effect or possibly a slightly injurious effect. Since blue occurs in high intensity in the mercury arc spectrum, it was used for reactivation in all succeeding experiments except in a few performed with the whole visible spectrum.

In all cases a lapse of time occurred between the time of irradiation and the time of photoreversal. Since in studies on bacteria it was found by Kelner (1949) and others (*e.g.*, Novick and Szilard, 1949) that the time during which photoreversal

can be achieved is relatively short at room temperature and in active cultures, the possibility arose that in the experiments reported here, the time between irradiation with UV and photoreactivation was too long. To determine whether any such loss in the capacity for photoreactivation occurred within a few hours following UV irradiation, illumination was performed at various times up to four hours after UV treatment. The amount of photoreactivation was observed to be even greater in some cases than if the blue light had been applied immediately after UV irradiation of colpidia [3] (Fig. 4). For the present series of experiments, it was considered quite adequate to perform the exposure to visible light within several hours of the ir-

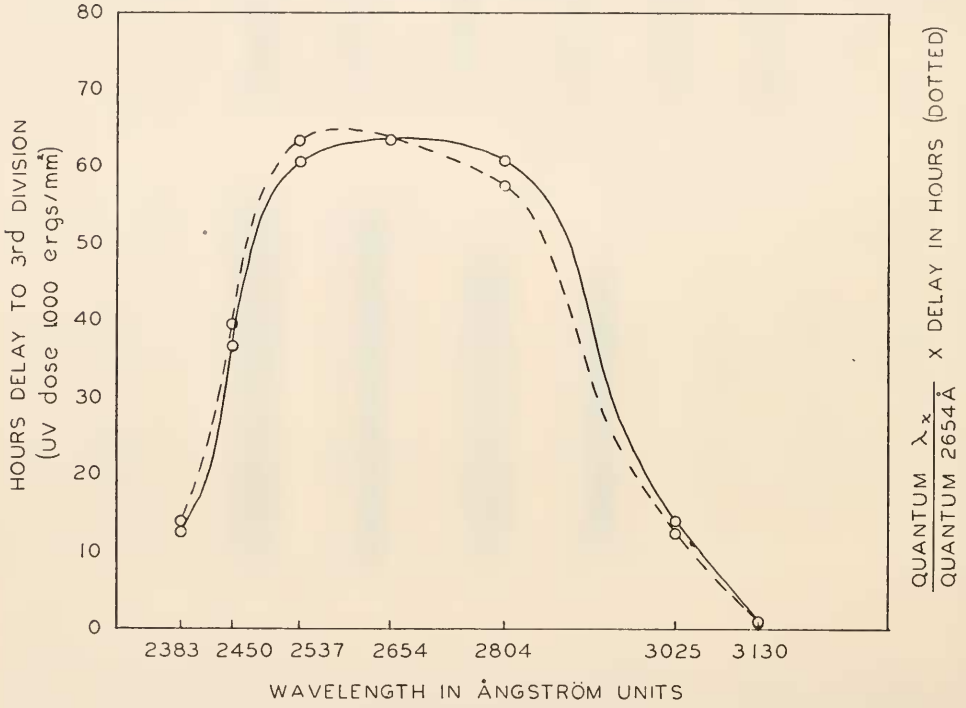

FIGURE 5. Relative efficiency of various wave-lengths of ultraviolet light upon division of Colpidium. The colpidia were irradiated with a dose of 1000 ergs/mm². at each of the wave-lengths. Comparison of relative efficiency on the basis of quanta is shown in the dotted curve.

radiation with UV. Because of the possibility of an increase in photoreactivation with lapse of time after UV exposure, it was necessary to illuminate with blue light at about the same time lapse after irradiation in order that the experiments might be comparable.

The final set of experiments was planned as a comparative study of the degree of photoreactivation following exposure at various UV wave-lengths. In most cases a dosage of 56,900 ergs/mm². of blue light (4350 Å) was used for photoreac-

[3] Recent experiments carried out by C. L. Brandt (unpublished) indicate that the reactivation can be achieved for as long as 23 hours after UV treatment, probably until the first division after UV treatment but not thereafter.

tivation. Figure 5 shows the relative efficiency of a dosage of 1000 ergs/mm². of each of the various UV wave-lengths used in retarding the division of Colpidium. The effectiveness is compared in two ways: 1) on the basis of the energy incidence per/mm²., and 2) on the basis of the number of quanta per mm². The results give a crude action spectrum for the UV retardation of division. The most effective wave-lengths are 2537, 2654, and 2804 Å. Wave-lengths on the short end: 2450 and 2383 Å, and on the long end: 3025 and 3130 Å, are less effective in retarding division than are the intermediate wave-lengths. In Figure 6 is shown the degree of photoreactivation produced by exposing to a dosage of 56,900 ergs/mm². at λ4350 Å colpidia previously treated with 1000 ergs/mm². at each of the UV wave-lengths.[4] Illumination in all cases was performed shortly after irradiation.

Pretreatment of colpidia with blue light (dosage: 46,000 ergs/mm².) does not protect them significantly from subsequent exposure to UV (2654 Å, 1000

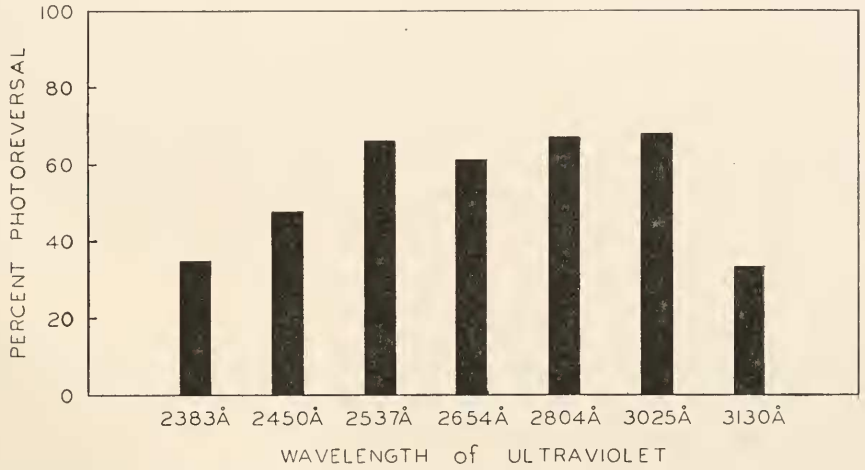

FIGURE 6. Degree of photoreactivation by blue light (56,900 ergs/mm².) after a dosage of 1000 ergs/mm². at each of the UV wave-lengths specified.

ergs/mm².). Colpidia so treated showed almost the same degree of retardation as those exposed to UV alone. Pretreatment with four hours of daylight before exposure to UV, however, produced some reduced sensitivity to UV applied thereafter. This phenomenon has been called *photodesensitization* by Weatherwax and Dobson (personal communication, 1951) who first observed it in *Escherichia coli* strain B. The decrease in sensitivity in Colpidium was only a small fraction of the photoreversal achieved by exposure to visible light after UV treatment.

DISCUSSION

UV affects division of Colpidium in two ways: a lag appears before division occurs, and, subsequent to larger doses, the rate of division is reduced. Treatment with visible light (or long UV) to a considerable extent reverses both of these ef-

[4] Colpidia treated with radiations of λ3025 Å were illuminated with only 42,000 ergs/mm². of blue light owing to an error in calibration, discovered on checking the calculation. This may introduce an error of several per cent in the value for this wave-length in Figure 6.

fects. UV treatment also caused a lag before division starts and a decrease in division rate in *Paramecium caudatum* (Giese, 1939) and in *Blepharisma undulans* (Giese and Reed, unpublished and Hirshfield and Giese, unpublished). In some ciliates, however, UV treatment also induced a cessation of division which occurs after one, sometimes after two, and, rarely, after three divisions have occurred subsequent to UV treatment. This was seen in *P. aurelia* and in *P. multimicronucleatum* (Giese and Reed, 1940; Kimball and Gaither, 1951). A similar effect has recently been found in *Tetrahymena geleii* (E. Christensen, unpublished), but it was never observed in Colpidium. The reason for this difference in effects of UV on different species of protozoa is not clear.

The different degrees of photoreactivation by blue light after irradiation of colpidia with various wave-lengths of the UV indicate that various UV wave-lengths may produce qualitatively different types of effects. This in turn may possibly be interpreted as being due to alteration of different chemical constituents of the cell by different wave-lengths. Thus certain wave-lengths may affect nucleoproteins more than other protoplasmic constituents, whereas other wave-lengths may selectively affect unconjugated proteins. This is most strikingly brought out (Fig. 6) by the relatively lesser degree of photoreactivation of UV injury at λ2383 Å, which produces rather superficial effects upon the colpidia, immobilizing them quite quickly. Immobilization of ciliary action in Paramecium shows an action spectrum with one maximum at λ2800 Å which corresponds to absorption by unconjugated proteins, whereas retardation of division of the cell shows an action spectrum with one maximum at λ2654 Å which corresponds to absorption by nucleic acid (Giese, 1945a). The results therefore suggest that visible light may be primarily concerned with reversal of UV damage to nucleoproteins or nucleic acid.

For photoreversal of UV injury in Colpidium, the most effective wave-lengths are similar to those found for *Streptomyces griseus* in which a maximum is found at λ4360 Å (Kelner, 1951). For *Escherichia coli,* and a phage which lives upon it, maximum photoreactivation is observed at about λ3650 to 3750 Å (Dulbecco, 1950; Kelner, 1951). Unfortunately the work on Colpidium is not precise enough to enable one to determine a detailed photoreactivation action spectrum. The main uncontrollable variable is the change in physiological state of the colpidia during the growth cycle. If one could obtain animals in exactly the same physiological state, this could be avoided. However, it is difficult to get experimental animals in exactly the same phase in the growth cycle since after a few divisions, the progeny of a single Colpidium begin to divide at a rhythm quite different from one another. The physiological state may have a profound effect on sensitivity to UV. Thus retardation of the third division of a vigorous group of colpidia (*e.g.,* those in which the three divisions occur within 20 to 22 hours in the control) by exposure to a dosage of 1000 ergs/mm². at λ2654 Å may be only 20 hours, whereas less vigorous colpidia (*e.g.,* those in which the three divisions occur in the control within 24 to 26 hours) given a similar dosage of UV may show a retardation of the third division of about two to three times the first group. Fortunately for experiments on photoreactivation, the dose reduction is similar; that is, the relative degree of recovery induced by visible light is similar regardless of the UV sensitivity of the colpidia. In recent experiments in which the colpidia were systematically starved before exposure to UV, great increases in sensitivity to UV have been observed with starvation

(Giese, Jacobson and Shepard, unpublished). The nature of the cellular change which causes such a striking difference in sensitivity has not been determined.

Since very large dosages of visible light are necessary for photoreversal of UV injury in Colpidium, it would appear that the substance or bond absorbing the visible light and bringing about the photoreversal is present in very small quantities or that the reaction is very inefficient because of the small amount of energy available in quanta of visible light. Since photodesensitization is so slight in Colpidium, it would appear likely that the compound concerned with photoreactivation appears in the form in which it can fruitfully absorb visible light only after absorption of UV. Shugar's studies (1951) suggest that one cellular compound absorbing light in the region of the spectrum effective in photoreactivation may be the enzyme D-glyceraldehyde-3-phosphate dehydrogenase, present with its reduced coenzyme. Absorption of the light by the adsorbed coenzyme results in reduction of the enzyme; light is ineffective if chemical reduction of the enzyme by cysteine is first performed.

Good photoreversal of UV injury in Colpidium by treatment with blue light even four hours after irradiation with UV light indicates that the injurious substance formed by UV light has a long life and that it does not exert its effect until some time after its production. At first sight this seems to be quite contrary to what has been found in bacteria (Kelner, 1949; Novick and Szilard, 1949). However, a normal Colpidium divides only about once every seven to eight hours at 26° C., whereas bacteria may divide every 30 to 60 minutes. When the inter-divisional time is large, the time during which photoreaction of UV injury can be achieved may also be long.

Summary

1. *Colpidium colpoda*, grown on a single strain of bacteria (*Pseudomonas ovalis*) in 0.05% lettuce medium buffered at pH 7, was irradiated with various wave-lengths of monochromatic ultraviolet (UV) light. UV treatment produces a characteristic lag before beginning of fission and, following larger dosage, a characteristic decrease in division rate. The greatest retardation of division was observed following exposure to λ2537, 2654 and 2804 Å.

2. The retarding effects of UV light at each wave-length were progressively greater with increasing dosages until division of treated colpidia was prevented. A dosage of 3000 ergs/mm². at λ2654 Å prevented division and resulted in death of 70 per cent of the colpidia.

3. Illumination with visible light of λ4350 Å effectively reversed UV injury resulting from a dosage of 1000 ergs/mm². at each of a number of wave-lengths of UV. For photoreactivation a dosage of 56,900 ergs/mm². of blue light (4350 Å) was used.

4. The degree of photoreversal by blue light of injury produced by wave-lengths 2537, 2654, 2804 and 3025 Å was larger than for wave-lengths 2383, 2450 and 3130 Å.

5. Pretreatment with blue light before exposure to UV light did not protect the colpidia; in other words a lag in, or retardation of, division comparable to that found for animals given the UV treatment alone was observed. However pretreatment with bright daylight protected colpidia to some extent from subsequent UV exposure.

6. Wave-lengths 3350, 3660 (long UV), 4050 (violet), and 4350 Å (blue) were effective in photoreversal of UV-injury in colpidia, but the yellow and green portions of the mercury arc spectrum were ineffective.

7. A large degree of photoreversal of UV-injury in colpidia could be induced even four hours after UV treatment, indicating persistence of the UV effect.

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